

Development of microsatellite markers to investigate recurrent polyploidy within the Genus  
*Dicentra*

By

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## ABSTRACT

The genus *Dicentra* (Bernhardi 1833) is a small group of herbaceous plants containing approximately 12 species found in North America and eastern Asia. Three species: *D. eximia*, *D. cucullaria*, and *D. canadensis*, display an interesting polyploid series in the Southern Appalachians, where *D. eximia* is a diploid ( $2n=16$ ), *D. cucullaria* is a tetraploid ( $2n=32$ ) and *D. canadensis* is an octoploid ( $2n=64$ ). To investigate recurrent polyploidy within the genus, a set of microsatellite markers are needed to assess whether populations are more closely related by ploidy level or geographic location. Many sequences generated from *D. cucullaria* were searched for microsatellite motifs. Fifty primer pairs were identified flanking various microsatellite motifs. These primer pairs were first tested on a small number of individuals including all three species to assess their ability to amplify a single locus. Those that successfully amplified across these samples were fluorescently labeled and submitted for genotyping, where allele size can be observed. Chromatograms were assessed for ease of scorability and allelic diversity. The markers verified in this study will be useful for investigating diversity and allow us to test the hypothesis of recurrent polyploidy.

## INTRODUCTION

Study group: Genus *Dicentra*

This study will focus on the biodiversity within the genus *Dicentra* (Bernhardi 1833). *Dicentra* traditionally belonged to the family Fumariaceae, based on irregular floral morphology and watery sap (Stern, 1961). Common floral morphologies throughout the genus are displayed as ultimate leaf segments that are 1-4 mm wide, basal leaf arrangements, and a corolla with the two outer petals spurred at their bases (Weakley, 2015). However, the Fumariaceae are often grouped with the Papaveraceae family and divided into subfamilies (Lidén 1981, 1986; Lidén et al. 1997; Judd, Sanders, & Donoghue 1994). Ultimately, recent molecular taxonomy has placed the genus in Papaveraceae (Weakley, 2015). The Papaveraceae are composed of approximately 23 genera and 230 species, mostly northern temperate herbs (Weakley, 2015). The genus *Dicentra* consists of about 12 perennial herbs species, three of which are pertinent to this study: *Dicentra eximia* ( $2n = 16$ ), *D. cucullaria* ( $4n = 32$ ), and *D. canadensis* ( $8n = 64$ ). Overall, *Dicentra* displays a relictual north temperate distribution, spanning through North America and East Asia (Weakley, 2015).

*Dicentra eximia* (Ker-Gawler) Torrey, is commonly known as the Bleeding Heart. The species displays a scaly and short rhizome, approximately 25-60 cm tall (Fig. 1). They have rigid pedicels that are approximately 2-10 mm long and sepals that are approximately 3-7 mm long. They display oblong, or lanceolate, ternately dissected leaves that are approximately 40 cm in length. They have petioles of approximately 10-40 cm long, with 6-12 dark pink flowers in lateral clusters over-topping the leaves. The two outer petals obtain rounded basal spurs 1-2 mm long. The style is approximately 8-10 mm long, displaying a capsule that is 16-25 mm long and has a diameter of 3-5 mm. *D. eximia* is commonly found on cliffs, talus slopes, rocky slopes,

rock outcrops, and shale slopes (Weakley, 2015). It can typically be observed blooming April through August (Weakley, 2015). As an Appalachian endemic species, *D. eximia* ranges from New York and New Jersey south through North Carolina, South Carolina, and Tennessee (Gaddy et al., 1984).



Figure 1. *Dicentra eximia*.

*Dicentra cucullaria* (Linnaeus) Bernhardt, is commonly known as Dutchman's Britches (Fig 2.). This species form from a short rootstock bearing small, tear-shaped, grain-like bulblets. The ternately decompound leaves are glaucous beneath and are broadly triangular in outline. There are generally 4-10 nodding flowers, with widely ovate sepals that are 2-4 mm long. The plant has a white corolla that is 15-18 mm long. The spurs located on the outer petals are 7-9 mm long and form a sagittate corolla base and the style is approximately 2-4 mm long. This species is commonly found in rich, moist forests, especially rich cove forests in the mountains (Weakley,

2015). It can be observed blooming March through June (Weakley, 2015). *D. cucullaria* ranges as far north as Minnesota and south to Georgia, Arkansas, and Kansas. Disjunct populations are also found in Washington, Oregon, and Idaho (Weakley, 2015).



Figure 2. *Dicentra cucullaria*.

*Dicentra canadensis* (Goldie) Walpers, is commonly known as Squirrel corn (Fig. 3). It forms from a short rootstock bearing small, yellow, pea-shaped bulblets. The leaves along the forest floor are glaucous beneath the stalk, broadly triangular in outline, and ternately decompose, it is 10-30 cm tall. This species displays slender petioles that are 8-20 cm long, sepals that are 2-5 mm long, a style that is 4-7 mm long, and a narrowly heart-shaped corolla that is often fragrant and 12-18 mm long. The flowers have short and rounded spurs, with a conspicuous, projecting crest of the inner petals. *D. canadensis* is often found occurring in



sympatry with *D. cucullaria*, residing in rich and moist forests, especially rich cove forests in the mountains (Weakley, 2015). It has been observed to bloom April through June (Weakley, 2015). *D. canadensis* ranges as far north as Minnesota, south to North Carolina, north Georgia, Tennessee, and Missouri (Weakley, 2015).



Figure 3.) *Dicentra canadensis*.

Each of the species included in this study are found in Eastern North America along the Appalachian Mountains, displaying ploidy numbers with a base chromosome number of 8 (Stern, 1968). *Dicentra eximia* has been described as a diploid species with  $2n = 16$ , *D. cucullaria* a tetraploid where  $2n = 32$ , and *D. canadensis* an octaploid where  $2n = 64$  (Fig. 4). Classically, species are described as monophyletic groups with a single origin. However, whole genome duplications (WGD) (or polyploidy) can result in the recurrent formation of a species (Soltis and Soltis, 1999). This phenomenon makes it possible that some populations of a species born

through WGD may represent independent duplication events. These events would generate populations that morphologically resemble each other, but are in fact derived multiple times. We argue the ploidy series seen in the genus *Dicentra* could represent a series of recurrent WGD events. As a result, we want to further investigate whether the polyploidy events of these species are of single origin or recurrent events. To do this, a set of microsatellite markers will be established to determine genetic variability amongst populations and to estimate genetic distance between populations of all three species.



Figure 4.) Display of chromosome in: a). *D. eximia* ( $2n = 16$ ) b). *D. canadensis* ( $2n = 64$ ) c). *D. cucullaria* ( $2n = 32$ ). (Hill, 1992).

### Polyploidy in plants:

Plant genomes vary in size and complexity, fueled in part by processes of whole-genome duplication, or polyploidization, and subsequent genome evolution (Servick et al., 2015). Despite repeated episodes of polyploidization throughout the evolutionary history of angiosperms, the genomes are not uniformly large, and even plants with very small genomes carry the signatures of ancient duplication events (Leitch and Bennett, 1997). The processes governing the evolution of plant genomes following these events are largely unknown. These processes contribute enormously to overall genetic variation throughout plant genomes and species (Alberts et al., 2002). With recent advancements in the study and knowledge of genetic materials, there are multiple ways in which one may describe variation amongst individuals.

According to Stebbins (1950), in *Variation and Evolution of Plants*, the ultimate sources of individual variation within plant genomes are mutations. Mayr uses an encompassing definition stating that, “a mutation is a discontinuous chromosomal change with a *genetic effect*” (as cited in Stebbins, 1950, p. 77). He used the word “discontinuous” to describe that these mutations produce small changes within the genome that leave little phenotypic impact, thus allowing them to blend with the continuous and fluctuating outcomes of individual variation (Stebbins, 1950). He also describes it as being “chromosomal”, meaning it is a chemical and structural change within a small part of the chromosome (Stebbins, 2015).

In general, mutations can be divided into four classifications: 1) multiplication of the entire chromosome set, or polyploidy; 2) addition or subtraction of one or a few chromosomes, or aneuploidy; 3) overall changes in the structure of the chromosome, including insertions, deletions, translocations, and inversions; and 4) submicroscopic changes, such as nucleotide substitutions, or small insertions and deletions within the DNA. This study will focus on the



processes of the first identified class of mutations, polyploidy. As the genus *Dicentra* displays geographically grouped ploidy series, the origin of this ploidy series will be investigated. Previously, it has been thought that most organisms have undergone polyploidy once, generating a new species of single origin. We propose that *Dicentra* represents a ploidy series that has not occurred once, but multiple times.

Polyploidy is described as one of the main mechanisms by which the genome has an increased number of genes, making it a very important aspect to the appearance and analysis of a genome (Van de Peer, 2017). To emphasize this point, Stebbins states, "...aside from polyploidy there is no evidence of a regular increase in the amount of chromosomal material during the evolution of the land plants" (Stebbins, 1950). In recent years, interpretations of plants as 'diploids' or 'polyploids' have been blurred, requiring much more nuanced vocabulary to describe plant genomes. As an extensively studied tool of genetic variation, polyploidy has become a commonly assessed indicator of genetic variation (Woodhouse et al., 2009).

In all groups of plants, polyploidy is known to occur to some degree, playing a greater or lesser role depending upon the clade or species examined. Stebbins describes that the distribution of polyploidy in vascular plants is practically spontaneous, and shows little to no direct correlation between chromosome number and phylogenetic position (Stebbins, 1950). He states, "most genera of angiosperms contain some polyploidy, most often in the form of tetraploid or hexaploid species, either scattered through the genus or concentrated in some of its sections" (Stebbins, 1950). The variability of polyploid multiples creates question of the polyploidization of the species. Stebbins discovered that the highest percentages of polyploids are found in perennial herbs, mostly located in temperate climates. Recent estimates suggest that 70% of all angiosperms have experienced one or more episodes of polyploidization (Soltis,

2009). With its major prominence known, polyploidy is central to understanding the evolution of angiosperms and other eukaryotic lineages (Leitch and Bennett, 1997). Along with this, discoveries state that transposable elements (TEs) may play a large role in genome reorganization after a polyploidization (Bennetzen, 2000). Transposable elements allow for easy removal of duplications due to polyploid events within the genome (Matzke and Matzke, 1995). These deletions cause rearrangements and shifts, resulting in an increase of genetic variation throughout the genomes within populations.

Generally, polyploidy as it occurs in nature is a product of either hybridization or a non-reduction event during meiosis. Two forms of polyploidy are found widely throughout polyploid species: autopolyploids and allopolyploids. There are two main types of polyploidy, but due the process of whole genome duplication and subsequent segmental loss, botanists describe four types: autopolyploids, segmental allopolyploids, true or genomic allopolyploids, and autoallopolyploids (Stebbins, 1950). Typical autopolyploids have now been produced artificially from many species, mostly of cultivated plants (Stebbins, 1949). The morphological difference between such polyploids and their diploid progenitors is remarkably close, with variations existing at physiological characteristics (Stebbins 1949).

Biologists typically argue that species are “born” from a single origin because of the close morphological structures between polyploids and the diploid progenitors. However, evidence is mounting that some polyploidy taxa may be formed multiple times, also known as recurrent polyploidy (Soltis and Soltis, 2009). “Polyploidy has played a major role in the evolution of many eukaryotes. Recent studies have dramatically reshaped views of polyploidy evolution, demonstrating that most polyploid species examined, both plant and animal, have formed recurrently from different populations of their progenitors” (Soltis and Soltis, 1999). Recurrent

polyploidy undergoes the same mechanism as autopolyploidy. Meaning, during meiosis a non-reduction event occurs and the chromosomes fail to separate properly during anaphase I. When this occurs, the genome of the organism undergoing meiosis doubles, resulting as a polyploid event (Soltis and Soltis, 2009). The traditional single origin autopolyploidy view differs from recurrent polyploidy in the idea that the revised view of recurrent polyploidy undergoes these non-reduction events in multiple independent origins, as opposed to a single event (Figure 5). This view has caused Soltis and Soltis to argue that “recurrent origins of polyploidy are the rule, not the exception” (1999).

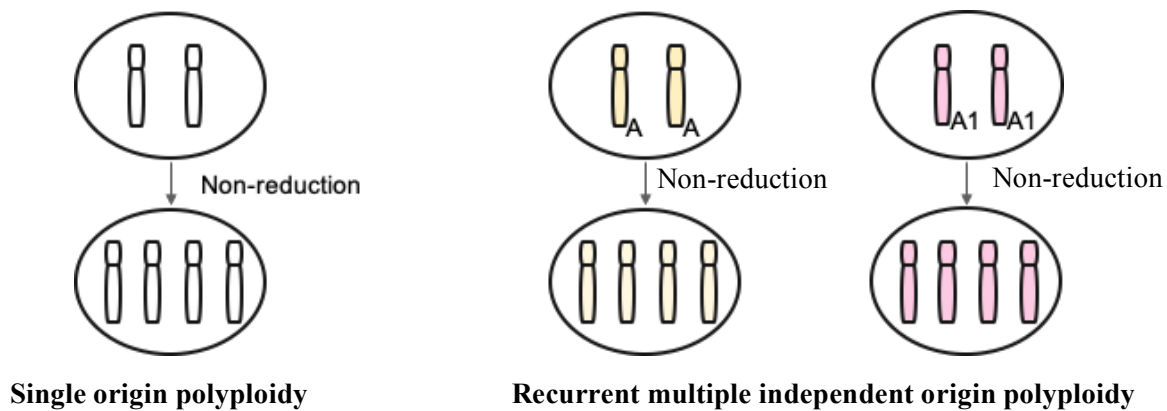


Figure 5. Schematic portraying how single origin and recurrent polyploidy undergo the same mechanism to achieved a genome duplication, but recurrent polyploidy happens in multiple independent origins. (Modified from Otto S. & Whitton J., 2000).

“Autopolyploids, as well as allopolyploids, are generally thought to form through the union of unreduced gametes (de Wet, 1980; Ramsey and Schemske, 1998, 2002); this process can take place either instantly through the fusion of two unreduced gametes, immediately generating an autotetraploid, or through the fusion of a single unreduced gamete and normal reduced gamete to produce an intermediate triploid” (Harlan and de Wet, 1975; reviewed by Ramsey and Schemske, 1998). One advantage described from the multiple independent origin hypothesis is that it would allow for higher amounts of genetic diversity in diploid progenitors to

be transferred to their polyploid gene pool (Otto, 2007). This may lead to the increased amount of genetic diversity and facilitate long-term survival and species adaptation in higher ploidy levels.

Traditionally, the formation of a new polyploidy species is thought of as a single event. This means the genetic diversity in each parent is all that is transferred to the new taxon. In some ways, this could be viewed as a founder effect, where a small amount of the total genetic diversity is used to initiate a new population. In recurrent polyploidy, many of the alleles present in the diploids can be passed to the new polyploidy taxa via multiple hybridization events. A complex of different alleles are created at the polyploid level when many genome multiplication events occur over genetically and morphologically differentiated diploid populations (Soltis and Soltis, 1999). These new polyploid taxa can be separated geographically, morphologically, and genetically, amongst other variables. Additional genotypes may be produced via gene flow through the polyploid series of independent origin (Soltis and Soltis, 1999). Gene flow between polyploid populations of independent origin might permit recombination of additional genotypes (Figure 6).

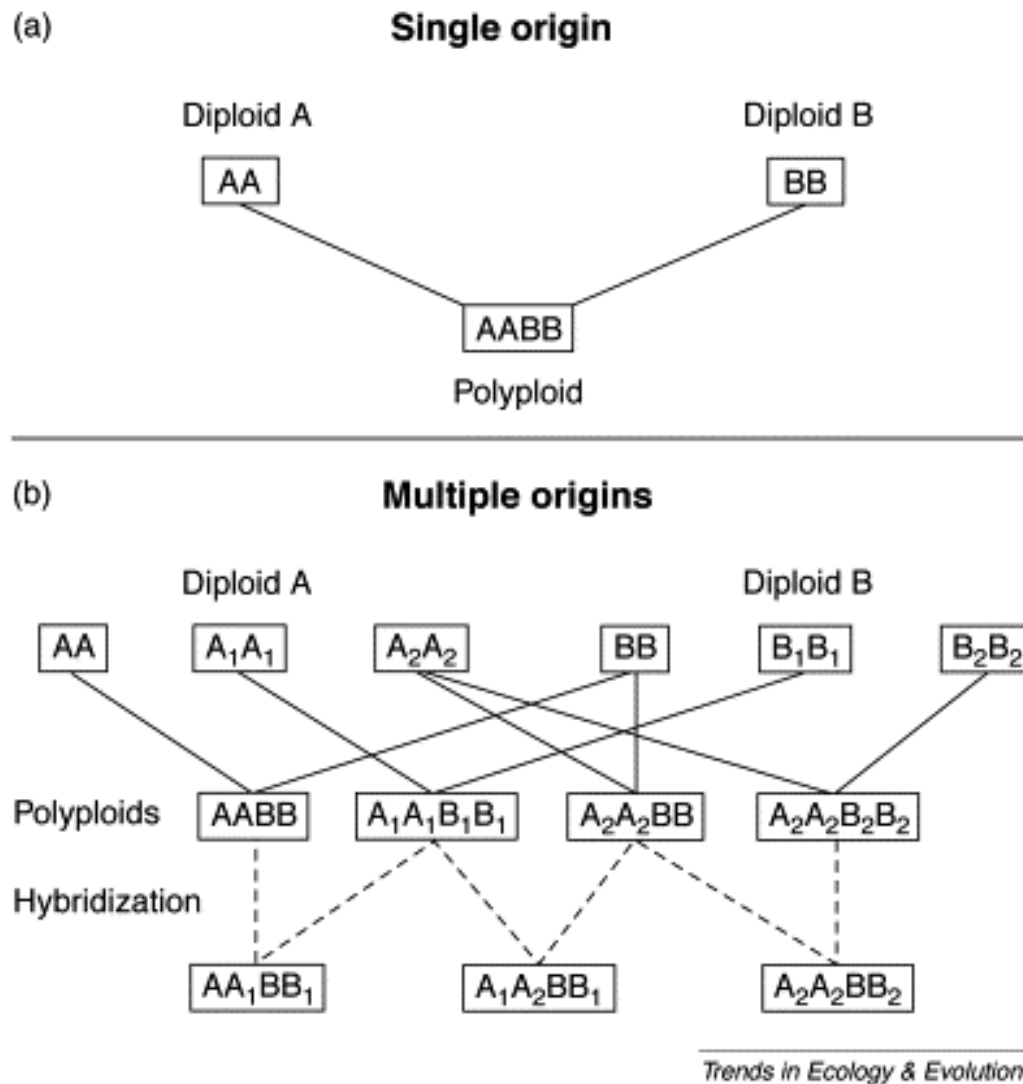


Figure 6. Comparison of the (a) traditional view of polyploid formation, single origin. This view shows that hybridization occurs once, forming genetically new progeny between different species. (b) Displays the revised version, that suggests that hybridization occurs many times, originating from different parental genotypes each time. The multiple origins view suggests that additional genetic variation is generated through a diverse array of recombination (Soltis and Soltis, 1999).

As stated previously, Soltis and Soltis explain that the evolution of plants and animals has occurred through the recurrent reproduction of the progenitors from different populations (Figure 6). This means that new genotypes can be formed by recurrent polyploidy (Soltis, 2015).

Populations of independent origin can come into contact and hybridize, generating new genotypes (Soltis and Soltis, 1999). They state a strong hypothesis saying recurrent origins of polyploid species are the rule, not the exception (Soltis and Soltis, 1999).

In support of this, the example of *Galax aphylla*, currently known as *Galax urceolata*, is now known to be of multiple independent origins (Servick et al., 2015). With this species belonging to a monotypic genus of the eastern United States which has no close relatives, the tetraploid differs in little besides its sturdier character and thicker leaves (Stebbins, 1980). As a long known polyploidy species, it was only recently discovered as undergoing recurrent polyploidy (Servick et al., 2015). Researchers found that the tetraploid species were often more closely related to diploids that were geographically close than other tetraploids. Higher levels of heterozygous genes than the diploids displayed, caused by up to 46 multiple polyploid events (Servick et al., 2015). These findings are among the highest frequencies of independent polyploidizations ever reported for any polyploid (Servick et al., 2015). It has about the same geographic distribution as the diploid, but its range is somewhat wider (Stebbins, 1980). Although the total number of recurrent polyploidy examples is limited, the actual extent of multiple origins for most polyploids is likely to have been underestimated (Soltis and Soltis, 1999). Other examples of recurrent polyploidy are *Tragopogon miscellus* and *T. mirus*. These species undergo hybridization, but are thought to have formed recurrently as many as 12-20 times, respectively, in only the past 60-70 years (Soltis and Soltis, 1999).

The genetic implications of recurrent polyploidy versus autopolyploidy, or single origin polyploidy, cause an interesting turn when studying polyploid species. Population-level genetic studies of polyploid plants and animals indicate that polyploidization should no longer be viewed as a rare event producing a polyploid species of unique origin and uniform genotype. Instead,



polyploid species can maintain high levels of segregating genetic variation through the incorporation of genetic diversity from multiple populations of their diploid progenitors. The recent discoveries of the origination and function of polyploidy have posed new questions to the community. It has allowed previously identified autopolyploid species to be reexamined for multiple independent origins, bringing to surface various variables of the plants genome.

The goal of this work is to sample populations of three *Dicentra* species and identify whether they are more closely related by ploidy level or geographic location. In order to do this a set of molecular markers must be constructed. Using highly variable molecular markers is critical for this study, as we must be able to compare closely related species. Thus, microsatellite markers will be used because they are efficient tools to provide links between phenotypic and genotypic variation (Varshney et al., 2005). Along with this, these markers are inexpensive to develop and are derived from sequence data, making them useful for assaying diversity in natural populations (Varshney et al., 2005). Microsatellites are generally one to ten base pair (bp) tandem repeats within the genomic repetitive regions (Vieira et al., 2016). These regions of the genome are susceptible to a higher mutation rate than other areas of DNA, leading to high genetic diversity (Vieira et al., 2016). Microsatellite markers are also highly variable within taxa and, ideally, neutral, meaning natural selection is not changing them. These markers will allow us to locate specific sequences of genetic material amongst the three species, observe which alleles are present, calculate varying allele frequencies, and determine heterozygosity within populations. Using this data, we can assess whether unique species are exchanging genetic information.

## METHODS

A total of 50 samples were collected in Boone, NC during April 2017 and Weaverville, North Carolina during April 2018 for all three taxa. Ten leaf tissue samples of cultivated *D. eximia* were collected from a local ornamental garden (constructed by Dr. Gary Walker) with unknown origin. Twenty samples for both *D. cucullaria* and *D. canadensis* were collected from a single location where both species are found in sympatry in Weaverville, North Carolina. Samples were only taken from plants that were flowering to be certain of which species were collected. The leaves were stored in individual 2 mL vials filled with approximately 1 mL of silica gel (Sigma-Aldrich International, Germany). After collections were complete, the silica gel vials holding the tissue were stored in the -80°C freezer until further analysis.

The tissue samples were ground to a fine powder using autoclaved sand and a mini pestle. Genomic DNA was extracted from the ground leaf tissue using a standard CTAB procedure (Doyle and Doyle, 1987). The resulting DNA was quantified using a Nanodrop 1000 (V3.6, Thermofisher, US) and imaged on a 1% agarose gel. The DNA was then diluted to 50ng/μL and was used to screen 50 selected microsatellite markers using polymerase chain reaction (PCR).

PCR master mix was prepared for 8 samples at a time using 2.0 μL of 5x GoTaq® Flexi Buffer, 1.0 μL of 25 mM MgCl<sub>2</sub>, 0.8 μL of 20 mM dNTP's, 0.5 μL of 10 mM forward primer, 0.5 μL of 10 mM reverse primer, 0.1 μL of 5u/μL GoTaq® DNA polymerase and 4.1 μL of 4.1M deionized water. A single experiment examined one primer pair against seven experimental samples (2 *D. eximia*, 2 *D. cucullaria*, 3 *D. canadensis*) and water (negative control). PCR was performed on an Eppendorf mastercycler (Eppendorf, Hauppauge, New York, USA) using Touchdown PCR conditions which entailed an initial 5 minute incubation at 94°C to denature the double stranded DNA, followed by 13 cycles at 94°C for 45 seconds, annealing starting at 68°C

and decreasing by 0.5°C per cycle for two minutes, followed by elongation at 72°C for 1 minute. The initial 13 cycles are then followed by 25 cycles at 94°C for 45 seconds, 50 °C for 1 minute, and 72°C for 1 minute. PCR is completed with a final extension at 72°C for 10 minutes. The PCR products were qualitatively analyzed using gel electrophoresis to ensure only one locus was amplified. Primers that did not amplify at all, amplified too many loci, or did not amplify all species, were eliminated from further marker development.

Twenty-four (48%) of the 50 primers tested were deemed successful in our initial screening. Those 24 markers were further examined across 8 individuals (2 *D. eximia*, 3 *D. cucullaria*, and 3 *D. canadensis*) and tagged with a florescent dye (VIC or FAM). Individual PCR products with different tags were then multiplexed into one plate using Hi-di and a LIZ 500 size standards (Applied Biosystems, US). PCR products were then sent to Georgia Genomics for separation of labeled fragments on an ABI3730 sequencer. The resulting chromatograms were then scored using Geneious 9.0.5 (Kearse et al., 2002) with the microsatellite plug-in (Kearse et al., 2002). A set of scoring standards were developed which included expected peak pattern, intensity of peak height, and the size range of the peaks.

## RESULTS

### Marker functionality:

Each marker was scored under a variety of parameters, including peak intensity, scorability of peak pattern, and the number of peaks produced per ploidy species. These qualitative measures were used to assess quality. Following the assessment, each marker was determined to be successful, in need of further refinement, or completely non-functional and removed from the study. (Table 1).

**Table 1. List of tagged microsatellite markers and functional selection.**

NAME	TAG	SUCCESS/FAILURE	REASON
D1	VIC	OTHER	Too many peaks in diploid
D2	FAM	FAILURE	Low amplification
D3	FAM	OTHER	Possibly monomorphic but unsure of pattern
D5	VIC	OTHER	Unable to score diploid
D6	VIC	OTHER	Possibly monomorphic and low amplification in diploid
D9	FAM	FAILURE	Too many peaks in diploid
D11	FAM	FAILURE	No product at all
D13	FAM	SUCCESS	Correct peak number, intensity, and scorable pattern
D15	VIC	FAILURE	Small products and too many peaks in all individuals
D16	FAM	FAILURE	No product at all
D17	FAM	OTHER	Unscorable peak pattern
D18	VIC	FAILURE	No product at all
D20	VIC	FAILURE	Low product amplification
D21	FAM	FAILURE	Low/no product amplification
D23	FAM	FAILURE	Too many peaks in all individuals
D24	VIC	FAILURE	Too many peaks in all individuals
D27	VIC	OTHER	Unsure of peak pattern
D28	VIC	SUCCESS	Monomorphic with correct peak number, intensity, and scorable peak pattern
D29	FAM	FAILURE	Too many peaks in all individuals
D34	VIC	OTHER	Unsure of peak pattern
D39	FAM	SUCCESS	Correct peak number, intensity, and scorable pattern
D40	VIC	OTHER	No amplification in diploid individuals
D49	VIC	SUCCESS	Correct peak number, intensity, and scorable pattern
D50	FAM	FAILURE	No amplification at all

Markers D13, D28, D39, and D49 were all considered functional as they displayed consistent peak patterns, the correct numbers of peaks per ploidy level, and high peak intensity (Fig 7). Marker D28 showed a consistent monomorphic pattern across each individual genotyped (Fig 7B). Marker D13 and marker D39 displayed a polymorphic peak pattern that was easily identified (Fig 7A and C). Marker D49 showed a polymorphic peak pattern that was variable, but displayed large intensities and the correct number of peaks (Figure 7D). Eight markers (D1, D3, D5, D6, D17, D27, D34, and D40) all displayed patterns that made scoring uncertain and difficult (Table 1). Markers D1, D5, and D6 displayed an unidentifiable peak pattern in the diploid species. Markers D3, D17, D27, and D37 had inconsistent peak patterns making them difficult to score. Marker D40 did not amplify in the diploid species. Twelve more markers (D2, D9, D11, D15, D16, D18, D20, D21, D23, D24, D29, and D50) were unscorable and removed from future analysis (Table 1).



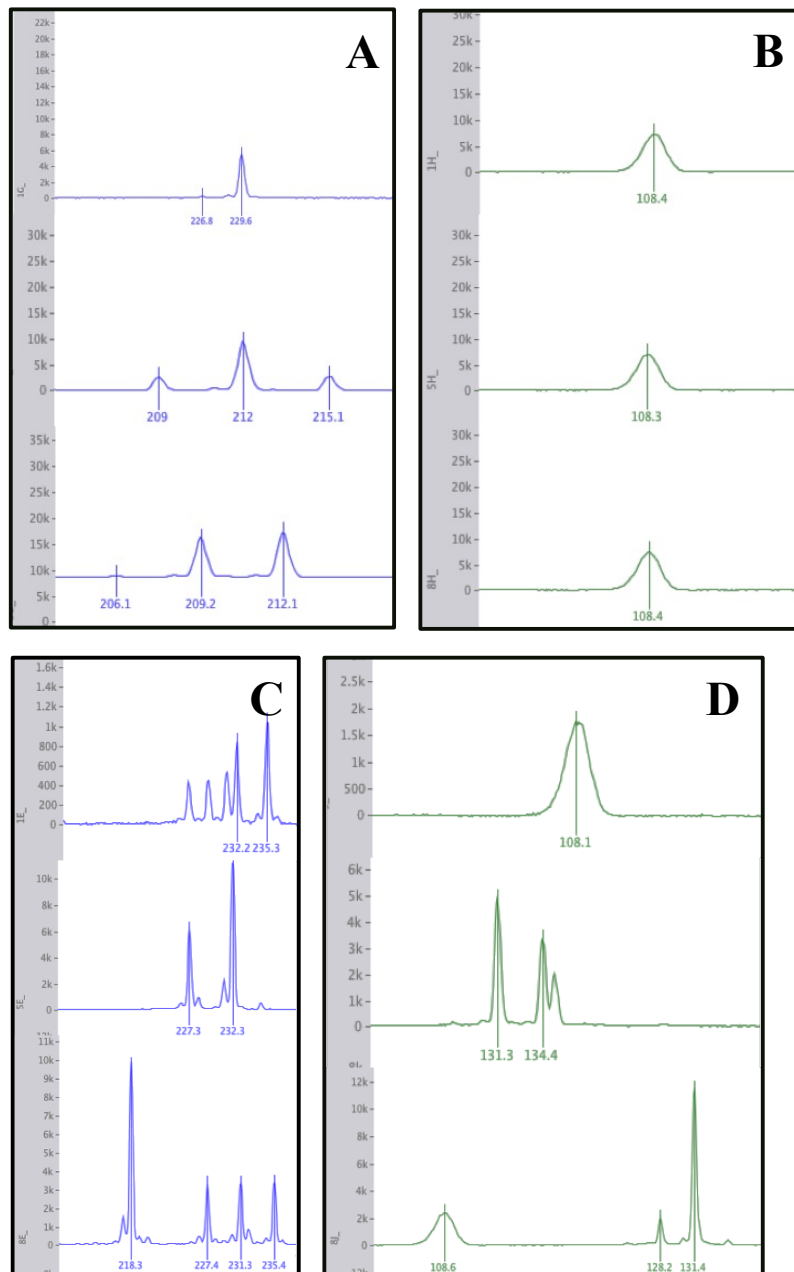


Figure 7.) Chromatograms displaying the four functional microsatellite markers. A.) Marker D13 shows polymorphic alleles with an identifiable peak pattern and correct number of peaks. B.) Marker D28 displays a monomorphic allele with a consistent peak pattern. C.) Marker D39 displays polymorphic alleles with consistent genetic variation. D.) Marker D49 shows polymorphic alleles with unidentifiable peak pattern, but good peak intensity and correct number of peaks.

Our analysis of possible markers results in four (8%) that produced successfully scorable chromatograms, eight (16%) that produced chromatograms that needed further evaluation, and twelve (24%) that produced chromatograms that failed to be scorable (Figure 8).

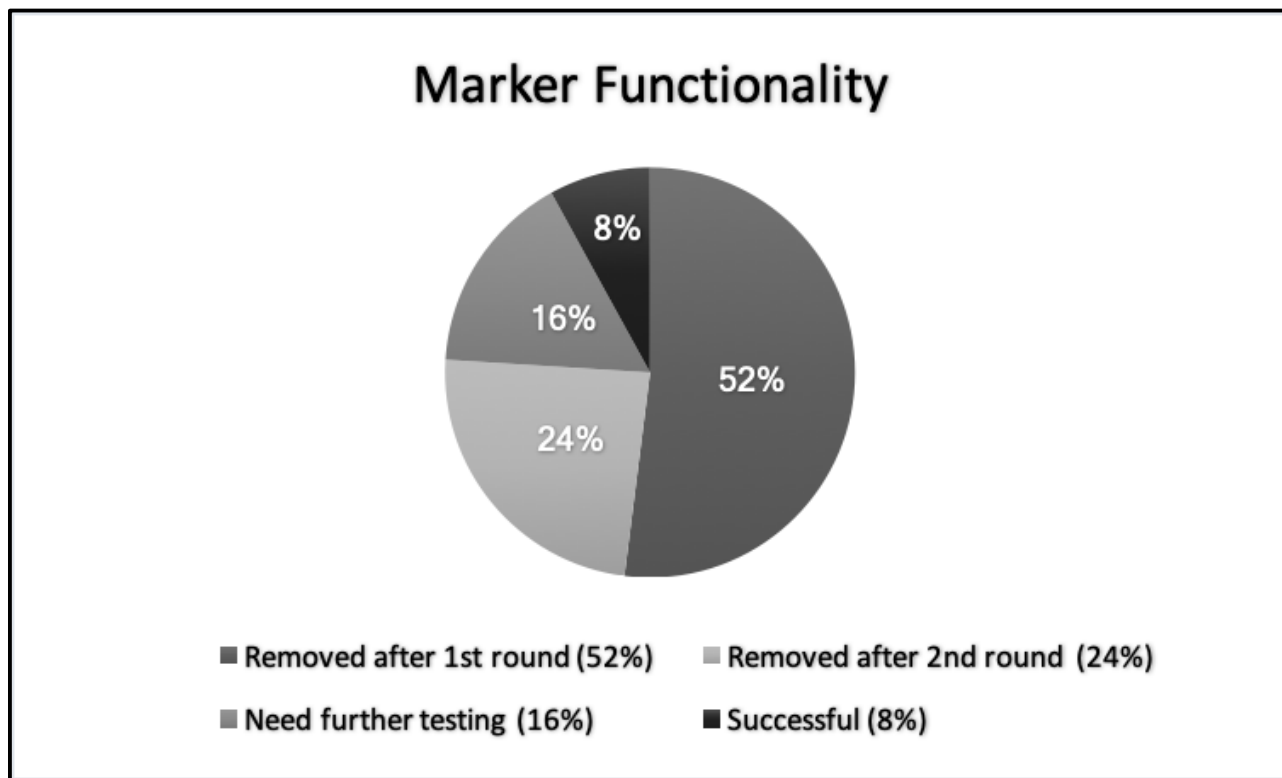


Figure 8.) Percentage of functional markers displayed in a pie chart. Of the original 50 markers, 52% were removed after the first round of testing, 24% were removed after the second round, 16% need further testing, and 8% were successful.

## DISCUSSION

*Dicentra* displays a ploidy series. These species are a small group of herbaceous plants that are found throughout the Appalachian Mountains in eastern North America. The purpose of this study was to develop genetic markers that could help identify if species within the genus were formed once or multiple times. To investigate whether these taxa represent single origin or recurrent origins, a set of microsatellite markers were developed.

A total of 50 primer pairs were selected for development. Twenty-four passed the 1<sup>st</sup> screening and were genotyped. Four of these were considered functional and will be used in future studies.

The four developed markers D13, D28, D39, and D49 were easily scorable, displayed strong PCR products, and showed some variability on a limited number of individuals. The remaining markers will not be useful in future studies.

A larger pool of microsatellite markers is needed to complete this study. Continued screening and validation of markers via PCR and chromatogram analysis is necessary. We would also like to employ a Mantel's test to measure whether geographic location of populations correlates with genetic diversity. Ideally, this measure should determine how closely related populations are based on genetic variation. This would show us if the populations of *Dicentra* are closely related genetically, or if they are simply distributed geographically close to one another. These findings would potentially allow us to answer the question: are ploidy species of *Dicentra* of single origin or multiple independent origins?

## CONCLUSION

In conclusion, we hypothesize that the ploidy species within the genus *Dicentra* undergo mechanisms of recurrent polyploidy. Due to the ploidy series observed and how often these species are found living in sympatry, we believe this study needs further investigation of the origins of polyploidy. Although the microsatellite markers displayed a low attrition rate, there were some that displayed genetic diversity within the parameters employed for functionality. This means further marker development and testing will be necessary. Once a suitable number of functional markers have been chosen, genetic diversity statistical analyses must be employed. This includes the employment of Mantel's test to measure the genetic variation in comparison to the geographic distance of the species. These analyses will determine whether the ploidy species of *Dicentra* have risen from a single origin or recurrent multiple independent origin polyploidy.

## BIBLIOGRAPHY

- Alberts B, Johnson A, Lewis J, et al. (2002). How Genomes Evolve. *Molecular Biology of the Cell. 4th edition.*
- Bennetzen, J. L. (2000). Transposable element contributions to plant gene and genome evolution. *Plant Molecular Biology*, **42**, 251-269.
- Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, **6**, 838-845.
- Hill, M. L. (1992). A floristic and chromosomal study of the Fumariaceae in Virginia. *Southern Appalachian Botanical Society*, **57**, 273-281.
- Hutchinson, J. (1921). The genera of Fumariaceae and their distribution. *Royal Botanic Gardens, Kew*, **3**, 97-115.
- Israilov, I., Melikov, F., Murav'eva, D. (1984). Alkaloids of *Dicentra*. *Chemistry of Natural Compounds*, **20**, 74-76.
- Leitch, I. and Bennett, M. (1997). Polyploidy in angiosperms. *Trends in Ecology and Evolution*, **2**, 470-476.
- Lidén, M. 1981. Proposal to change the typification of *Corydalis* nomen conservandum. *Taxon* **30**, 323-325.
- Lidén, M. 1986. Synopsis of Fumarioideae (Papaveraceae) with a monograph of the tribe Fumarieae. *Opera Botanica* **88**, 1-133.

- Lidén, M., T. Fukuhara, J. Rylander, and B. Oxelman. 1997. Phylogeny and classification of Fumariaceae, with emphasis on *Dicentra* s.l., based on the plastid gene rps16 intron. *Pl. Syst. Evol.* **206**, 411-420.
- Masterson, J. (1994). Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science*, **264**, 421-423.
- Matzke, M. and Matzke, A. (1995). Why and how do plants inactivate homologous (trans)genes? *Plant Physiology: American Society of Plant Biologists*, **107**, 679-685.
- Otto, S. P. (2007). The evolutionary consequences of polyploidy. *Cell*, **131**, 452-462.
- Panawala, L. (2017). Difference Between Autopolyploidy and Allopolyploidy. *PEDIAA*, 1-7.
- Pérez-Gutiérrez, M.A., A.T. Romero-García, M.J. Salinas, G. Blanca, M.C. Fernández, and V.N. Suárez-Santiago. (2012). Phylogeny of the tribe Fumarieae (Papaveraceae s.l.) based on chloroplast and nuclear DNA sequences: evolutionary and biogeographic implications. *American Journal of Botany*, **99**, 517-528.
- Pires, J., Yoong Lim, K., Kovarik, A., Matyasek, R., Boyd, A., Leitch, A., Letich, I., Bennett, M., Soltis, P., and Soltis, D. (2004). Molecular cytogenetic analysis of recently evolved *Tragopogon* (Asteraceae) allopolyploids reveal a karyotype that is additive of the diploid progenitors. *American Journal of Botany*, **91**, 1022-1035.
- Servick, S., Visger, C., Gitzendanner, M., Soltis, P., and Soltis, D. (2015). Population genetic variation, geographic structure, and multiple origins of autopolyploidy in *Galax urceolata*. *American Journal of Botany*, **102**, 973-982.



- Soltis, P., Marchant, D. B., Van de Peer, Y., Soltis, D. (2015). Polyploidy and genome evolution in plants. *Current Opinion in Genetics and Development*, **35**, 119-125.
- Soltis, P., Soltis, D., Pires, J. C., Kovarik, A., Tate, J. A., Mavrodeiv, E. (2004). Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic, and genetic comparisons. *Biological Journal of the Linnaean Society*, **82**, 485-501.
- Soltis, D. and Soltis, P. (1999). Polyploidy: recurrent formation and genome evolution. *Trends in Ecology and Evolution*, **14**, 348-352.
- Stebbins, G. L. Jr. (1949). The evolutionary significance of natural and artificial polyploids in the family of *Gramineae*. *University of California, Berkeley*.
- Stebbins, J. L. (1980). Polyploidy in plants: unsolved problems and prospects. *Polyploidy. Basic Life Sciences*, **13**, 495-520.
- Stern, K. (1961). Revision of *Dicentra* (Fumariaceae). *Brittonia*, **13**, 1-57.
- Van de Peer, Y., Mizrachi, E., Marchal, K. (2017). The evolutionary significance of polyploidy. *Nature Reviews Genetics*, **18**, 411.
- Varshney, R., Graner, A., Sorrells, M. (2005). Genetic microsatellite markers in plants: features and applications. *Trends in Biotechnology*, **23**, 48-55.
- Vieira, M., Santini, L., Diniz, A., Munhoz, C. (2016). Microsatellite markers: what they mean and why they are so helpful. *Genetics and Molecular Biology*, **39**, 312-328.

- Wang, W., A.-M. Lu, Y. Ren, M.E. Endress, and Z.-D. Chen. (2009). Phylogeny and classification of Ranunculales: evidence from four molecular loci and morphological data. *Perspectives in Plant Ecology, Evolution and Systematics*, **11**, 81-110.
- Wada, Y., Kaga, H., Uchiito, S., Kumazawa, F., Tomiki, M., Onozaki, Y., Nobuhito, Kurono, Tokuda, M., Ohkuma, T., and Orito, K. (2007). On the synthesis of protopine alkaloids. *The Journal of Organic Chemistry*, **72**, 7301-7306.
- Weakley, A. (2015). Flora of the Southern and Mid-Atlantic states. University of North Carolina Herbarium, North Carolina Botanical Garden, University of North Carolina, Chapel Hill.
- Whittaker, J., Harbord, R., Boxall, N., Mackay, I., Dawson, G., Sibly, R. (2003). Likelihood-based estimation of microsatellite marker mutation rates. *Genetics*, **164**, 781-787.
- Woodhouse, M., Burkart-Waco, D. & Comai, L. (2009). Polyploidy. *Nature Education*, **2**, 1.